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The Number of CGG Repeats of the FMR1 Locus in Premutated and Fully Mutated Heterozygotes and Their Offspring: Implications for the Origin of Mosaicism

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The size of the CGG repeat of the FMR1 gene was investigated with probe *StB12.3* in 154 transmissions to the offspring of heterozygotes for the premutation and the full mutation. Among the 135 offspring of premutated heterozygotes there were three decreases in size of the repeats: in two of these cases a full mutation was present along with the decreased premutation, and in a third mosaic (46,fra(X)(q27.3),Y), a normal allele was observed. In the 19 offspring of fully mutated females with no detected mosaicism, there were three mosaics and three individuals who had full mutations that included a number of repeats smaller than those present in their mothers. Among the 32 offspring who received a premutation from their premutated mothers, 27 alleles were increased in size and 5 remained unaltered. Among 11 mosaic offspring of premutated mothers, the premutation increased in 4, decreased in 3, and was unchanged in 4. In contrast to the trend of an increasing premutation size in the non-mosaic offspring, the premutation present in mosaics can be smaller, larger, or of unaltered size with approximately equal frequencies. These data suggest that the premutations present in mosaics result from mitotic instability of the inherited full mutations. This is further supported by the finding of a mosaic male with a normal sized allele. © 1996 Wiley-Liss, Inc.

KEY WORDS: fra(X) mosaics, fra(X) syndrome, probe *StB12.3*

INTRODUCTION

Fragile X syndrome is one of the most frequent inherited forms of mental retardation. It results from mutations in the CGG repeat sequence in the first exon of the FMR1 gene [Oberlé et al., 1991; Verkerk et al., 1991]. This CGG repeat is polymorphic in the normal population and varies in size from six to 52 repeats. Repeats within this range have never been found to expand when transmitted, indicating their stable nature. In the phenotypically normal carriers of fragile X premutations, the numbers of CGG repeats are expanded up to 200 repeats (Δ = 50–500 bp). Larger expansions are present in the fully mutated alleles. Affected individuals always have a full mutation that may be observed in combination with a premutation (mosaicism). Premutations tend to expand when transmitted to offspring through female carriers and the risk of expansion to a full mutation correlates with the size of the premutation [Fu et al., 1991; Heitz et al.,1992].

Decreases in the size of the CGG repeat is much less frequent. In some instances the size of the full mutation was observed to be smaller in the offspring of fully mutated mothers [Oberlé et al., 1991; Snow et al., 1993; Chiurazzi et al., 1994]. Contraction of a full mutation to a premutation has also been reported both in premutated [Fu et al., 1991; Oberlé et al., 1991] and mosaic offspring [Snow et al., 1993; Chiurazzi et al., 1994]. A smaller premutation has been described in the son of a premutated mother by Chiurazzi et al. [1994]. In some rare instances a normal allele has been found in a male patient along with a full mutation [Snow et al., 1993; van den Ouweland, 1994a]. More recently, the contraction of a premutation to a normal allele was described in the daughter of a carrier female [van den Ouweland et al., 1994b; Vits et al., 1994]. Based on linkage studies, van den Ouweland et al. [1994b] concluded that gene conversion was the mechanism involved in their case.

Whether a premutation or a full mutation is present in the oocytes of females carrying a full mutation in their blood cells is not known. There is the possibility that they are mosaics that always transmit the unstable premutation giving rise to the full mutation in the embryo. In this respect, the finding of only a premuta-

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TABLE I. Comparison of the CGG Repeat Length of the FMR1 Gene Between Heterozygotes for the Fragile X Mutation and Their Offspring*

Heterozygotes	Offspring									
				Fully mutated						
	Premutated Δ			Mosaic Δ			Non mosaic Δ[a]			
	>	<	=	>	<	=	>	<	=	Total
Premutated (n = 74)	27		5	4	3	4				
	32			11			92			135
Fully mutated (n = 14)				3[b]			9	3	4	19
							16			
88	32			14			108			154

*Δ: > increased, < decreased, = unaltered in relation to maternal Δ.
[a]The smallest Δ.
[b] Full mutations with larger Δ.

tion in sperm cells of fully mutated males is noteworthy [Reyniers et al., 1993]. On the other hand, our data show that in the mosaic offspring of premutated mothers the size of the premutation may increase, decrease or be unaltered with about equal frequencies, contrasting with the trend of an increasing size of the premutation in the premutated offspring. This suggests that the premutations present in mosaics result from mitotic instability of the full mutations.

SUBJECTS AND METHODS

A total of 88 heterozygotes and their 154 offspring were studied. The families were mostly ascertained through a mentally retarded individual.

DNA samples extracted from whole blood were double digested with *EcoR I* and *Eag I* or *Bgl II* and probed with *StB12.3* (a kind gift of F. Rousseau), as previously described [Mingroni-Netto et al., 1994]. PCR amplification was performed according to Haddad et al. [in press]. In brief, PCR amplification of a product of 557 bp for the $(CGG)_{29}$ allele is accomplished with primers that flank the trinucleotide repeats. Conditions are such that full mutations fail to amplify. A third primer allows the amplification of a 223 bp monomorphic internal control fragment. The amplified fragments were analyzed on a silver-stained non-denaturing polyacrilamide gel [Santos et al., 1993].

RESULTS

The lengths of the CGG repeats of the FMR1 gene, as determined by Southern blotting, were compared between heterozygotes and their offspring. Data are summarized in Table I.

Offspring of Premutated Heterozygotes

Among 32 premutated offspring, 27 alleles were expanded in relation to those present in mothers and 5 remained unaltered. The 103 fully mutated offspring included 11 mosaics. The premutation had the same size as that present in the mother in four of these mosaics, increased in four and decreased in three (Fig. 1). In one of the latter patients, an allele of normal size was observed (Fig. 2). He had a normal XY constitution in 100 peripheral blood lymphocytes and expressed fra(X) with a frequency of 40%. The normal range size of one of his alleles was demonstrated by Southern blotting after EcoRI/EagI (Fig. 2A) and BglI (Fig. 2B) digestions. PCR amplification yielded a normal sized product of about 492 bp (Fig. 2C).

Offspring of Fully Mutated Heterozygotes

All the 19 children of 14 fully mutated heterozygotes carried a full mutation. Three of them were mosaics

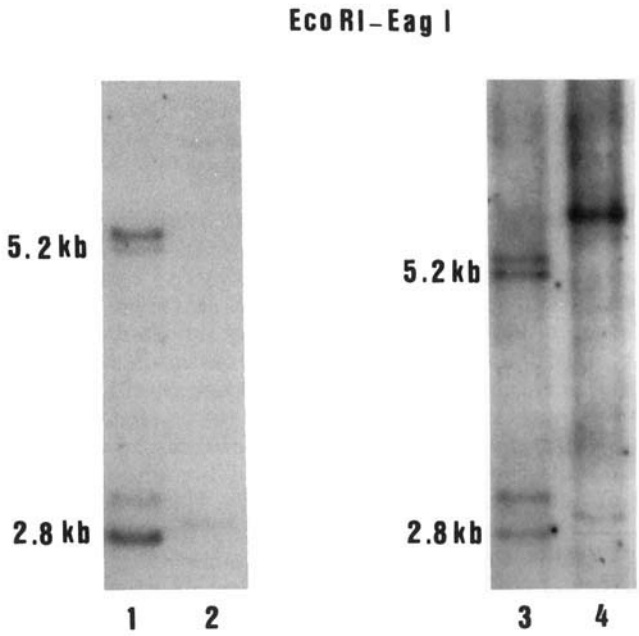

Fig. 1. Premutated heterozygotes with Δ = 200 bp (**lanes 1** and **3**) and their affected mosaic sons (**lanes 2** and **4**) who carry unmethylated alleles with Δ = 100 bp.

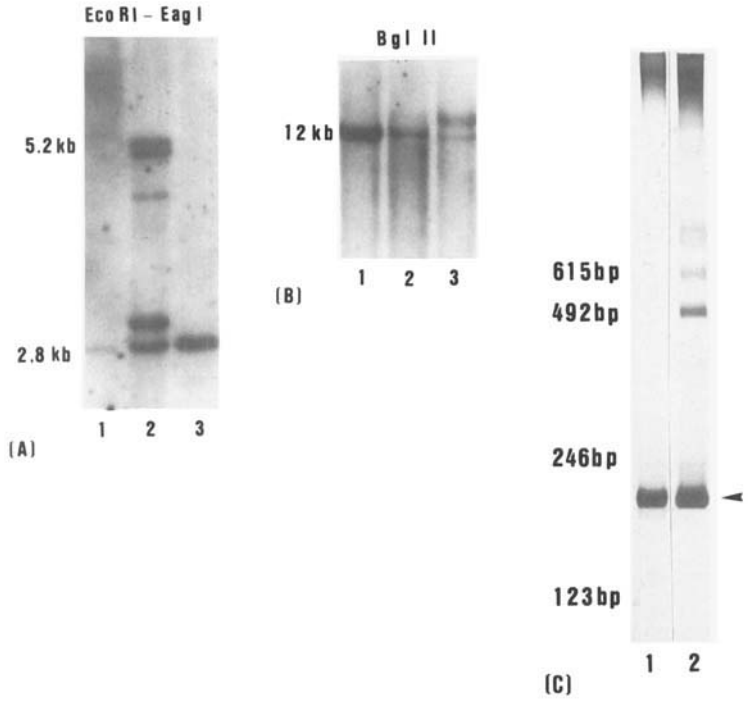

Fig. 2. A normal sized allele in a mosaic male. *StB12.3* hybridization. **A:** *EcoR I/ Eag I* digests: affected mosaic male with the unmethylated normal sized allele (**lane 1**), his premutated mother with Δ = 200 bp (**lane 2**) and a normal male (**lane 3**); the dark spot in lane 2 in the range of an unmethylated expansion is an artifact (demonstrated in other blots not shown here). **B:** *Bgl II* digests: the normal and mutated alleles of the same patient (**lane 3**) and the normal pattern of two control females (**lanes 1** and **2**). **C:** A silver-stained non-denaturing gel showing the pattern of PCR amplification of a CGG repeat containing segment and of a monomorphic fragment of 223 bp, the internal control. This internal control (arrow) is the only fragment that is amplified in a nonmosaic fully mutated male (lane 1); in the mosaic patient, a segment including the CGG repeat is also amplified as a fragment in the normal size range (approximately 492 bp).

and in these the full mutations were larger than the maternal ones. In the 16 nonmosaic cases, three had expansions smaller than those of their mothers (Fig. 3). In the remaining 13, at least one allele (the smallest) was expanded (9 cases) or unaltered (4) in size when compared to those of the mothers.

DISCUSSION

The trend for the expansion of the fragile X premutation when transmitted through female meiosis has been documented since the cloning of FMR1 gene [Oberlé et al., 1991; Fu et al., 1991]. However, it remains to be clarified whether fully mutated offspring receive from their mothers the full mutation or the premutation that subsequently undergoes amplification to a full mutation. This question was raised since mosaics were first reported and was further discussed when the full mutation was shown not to be transmitted by fully mutated males [Willems et al., 1992; Hori et al., 1993].

When we analyzed the offspring of premutated heterozygotes, we observed that the premutation was increased in 78% (27/32) of premutated children and was unaltered in the remainder. On the other hand, when the premutation was present along with a full mutation, it appeared expanded (4/11), contracted (3/11), or similar to the maternal one (4/11) with about equal frequencies. This different pattern does not correlate to the size of the premutation in the heterozygotes, since the mean maternal did not differ for the groups of mosaic and nonmosaic children ($t = 0.0125$; $P = 0.397$). An explanation for this would be that the premutations in mosaics do not represent the inherited alleles, but otherwise result from the mitotic instability of the full mutation. This instability of fully mutated alleles is clearly documented by the common observation of full mutations of different sizes, frequently visualized as a smear, when the DNA of fully mutated individuals is investigated in Southern blots. On the other hand, premutations appear somatically stable, and smears in the premutation range are very rarely described [Fu et al., 1991; Rousseau et al., 1994]. If a premutation originated from a fully mutated allele, it would be stably transmitted to daughter cells. This would be a rarer mitotic event than those generating slight differences in size of fully mutated alleles. This mechanism would give rise to individuals with somatic mosaicism, which occur much less frequently than those carrying only fully mutated alleles [Snow et al., 1993; Rousseau et al., 1994].

Mosaics with an allele in the normal range coexisting with a full mutation are noteworthy in demonstrating the mitotic origin of the unmethylated allele in mosaics, since a normal sized inherited allele does not seem to be able to amplify directly to a full mutation. Besides the patient here described, such mosaics were

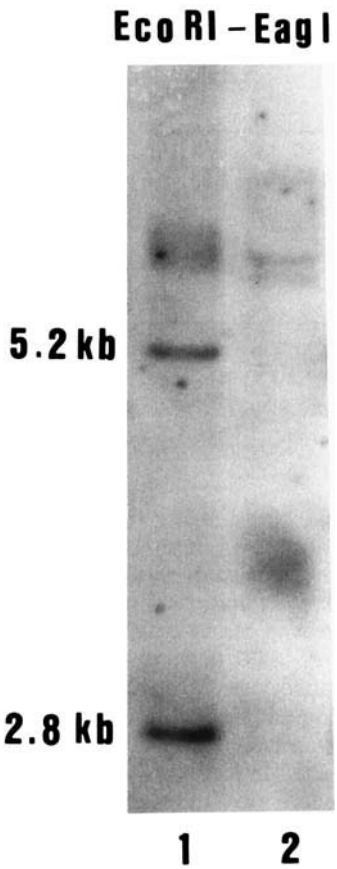

Fig. 3. Affected male (**lane 2**) with fully mutated allele smaller than those detected in his mother (**lane 1**).

reported by Snow et al. [1993] and van den Ouweland et al. [1994a]. These cases, along with those four mosaics with alleles smaller than the normal range, that indicated a hot spot for deletions in the CGG containing region [Graaff et al., 1995], make it more tempting to speculate that mosaics result from deletions in the unstable full mutation.

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